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Differential Expression of Two Endo-1,4-β-Glucanase Genes in Pericarp and Locules of Wild-Type and Mutant Tomato Fruit[1]

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The mRNA accumulation of two endo-1,4-β-D-glucanase genes, *Cel1* and *Cel2*, was examined in the pericarp and locules throughout the development of normal tomato (*Lycopersicon esculentum*) fruit and the ripening-impaired mutants *rin* and *Nr*. Both *Cel1* and *Cel2* were expressed transiently at the earliest stages of fruit development during a period corresponding to cell division and early cell expansion. In the pericarp, the mRNA abundance of both genes increased markedly at the breaker stage; the level of *Cel1* mRNA decreased later in ripening, and that of *Cel2* increased progressively. *Cel2* mRNA levels also increased at the breaker stage in locules but after initial locule liquefaction was already complete. In *rin* fruit mRNA abundance of *Cel1* was reduced and *Cel2* was virtually absent, whereas in *Nr* *Cel1* was expressed at wild-type levels and *Cel2* was reduced. In wild-type fruit ethylene treatment slightly promoted the mRNA accumulation of both genes. In *rin* fruit ethylene treatment strongly increased the mRNA abundance of *Cel1* to an extent greater than in wild-type fruit, but *Cel2* mRNA was absent even after ethylene treatment. These two endo-1,4-β-D-glucanase genes, therefore, do not show coordinated expression during fruit development and are subject to distinct regulatory control. These results suggest that the product of the *Cel2* gene contributes to ripening-associated cell-wall changes.

The firmness of developing tomato fruit begins to decline at the onset of ripening, at approximately the BR stage, when the first sign of red color becomes evident, and then decreases progressively (Sobotka and Watada, 1971; Maclachlan and Brady, 1994). During ripening both cell-wall pectins and cell-wall hemicelluloses undergo degradation (Huber, 1983). Increases have also been measured in the activity of polysaccharide-degrading enzymes, including PG (Gross and Wallner, 1979), EGase (Hobson, 1968; Babbitt et al., 1973), and numerous glycosidases (Wallner and Walker, 1975; Pharr et al., 1976; Pressey, 1983; Carey et al., 1995). Although PG expression (DellaPenna et al., 1986) and activity (Brady et al., 1982) are temporally correlated with fruit softening, PG-mediated pectin breakdown alone is not sufficient to cause it (Sheehy et al., 1988; Smith et al., 1988; Giovannoni et al., 1989). Other cell-wall hydrolases may therefore make important contributions to the extensive cell-wall disassembly that occurs during fruit softening. One class of cell-wall hydrolases, cellulases or EGases (EC 3.2.1.4), has been specifically implicated in a number of developmental processes, including cell expansion (Hayashi and Maclachlan, 1984) and organ abscission (Sexton and Roberts, 1982), as well as ripening-associated fruit softening (Hall, 1964; Hobson, 1968; Christoffersen et al., 1984).

An increase in extractable carboxymethylcellulase activity during tomato fruit ripening has been described in many studies (Hobson, 1968; Babbitt et al., 1973) and can be correlated with a decrease in the molecular size of cell-wall hemicelluloses (Huber, 1983; Tong and Gross, 1988), including xyloglucan (Sakurai and Nevins, 1993; Maclachlan and Brady, 1994). Extractable carboxymethylcellulase activity is presumably due to EGases, which in tomato are encoded by a multigene family (Brummell et al., 1994; Lashbrook et al., 1994). cDNA clones have been isolated for two tomato EGases *Cel1* and *Cel2*, in which expression increases during flower abscission and fruit ripening (Lashbrook et al., 1994). In fruit pericarp, expression of both of these genes was greatly induced at the BR stage, with mRNA levels of *Cel2* eventually reaching up to a 4-fold greater abundance than those of *Cel1*. Treatment of fruit with the ethylene action antagonist 2,5-norbornadiene reduced the mRNA abundance of both of these genes to low or nondetectable levels (Lashbrook et al., 1994), suggesting that their expression is ethylene dependent.

The present study was designed to further characterize the roles of *Cel1* and *Cel2* in fruit ripening. To test whether the *Cel1* and *Cel2* genes are coordinately regulated and to evaluate the potential contribution of each gene product to the ripening process, expression analysis was extended to include the earliest stages of fruit development, including cell division. We also examined the effects of exogenous ethylene treatment on the expression of these genes during fruit ripening. Expression of *Cel1* and *Cel2* was also examined in fruit locules, which exhibit greater extractable EGase activity than does pericarp (Huber, 1985; Maclachlan and Brady, 1992), and in fruit of the tomato

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Abbreviations: BR, IG, LR, MG, PK, RR, TU, breaker, immature green, light red, mature green, pink, red ripe, and turning tomato fruit developmental stages, respectively; EGase, endo-1,4-β-D-glucanase; *Nr*, Never ripe mutant of tomato; PG, endopolygalacturonase; *rin*, ripening inhibitor mutant of tomato.

ripening-impaired mutants *rin* and *Nr* (Tigchelaar et al., 1978).

MATERIALS AND METHODS

Green fruit were collected from field-grown plants of tomato (*Lycopersicon esculentum* Mill. cv Castlemart) either for immediate freezing or for subsequent ripening off the vine. Young fruit were staged by size and internal anatomy, and fruit at the youngest stages (5, 10, and 20 mm in diameter) were frozen directly in liquid N_2. Three fruits from each of stages IG1 to MG4 (defined below) were sectioned, and the locular contents and pericarp were separated and frozen in liquid N_2 and stored at −80°C. MG fruit were ripened at 25°C in 22-L glass chambers aerated at a flow rate of 20 L h^{-1} with either humidified air alone or with humidified air containing 10 μL L^{-1} ethylene. Fruit were staged by color and ethylene production (Lashbrook et al., 1994) into defined ripening stages as follows: IG1, fruit approximately 25% of fresh weight of MG, seeds immature; IG2, 50% of fresh weight of MG; IG3, 75% of fresh weight of MG; MG1, green fruit, locules solid but seeds fully developed; MG2, one or two locules liquefied; MG3, all locules liquefied; MG4, slight pink coloration to locules, no exterior color; BR, slight red color visible on exterior of fruit at blossom end; TU, fruit 10 to 30% light red; PK1, fruit 30 to 50% light red; PK2, fruit 50 to 70% light red; LR, fruit 100% light red; RR, fruit dark red and table ripe. Three fruits per stage were separated into locular contents and pericarp, frozen in liquid N_2, and stored at −80°C.

Tomato plants of cv Ailsa Craig either wild type or nearly isogenic for the *rin* or *Nr* ripening mutations were grown in the greenhouse. Flowers were tagged as the swelling ovary reached 2 to 3 mm in diameter, and fruit were collected 35 d later (T0, equivalent to late MG stage). Wild-type *rin* and *Nr* fruit at 35 d after anthesis were nearly identical in size, color, morphology, and locular development, indicating that their development prior to ripening was similar and that the selected time represented a comparable physiological stage. Five fruits per treatment time were either separated into locules and pericarp and frozen immediately as above or ripened in air or in ethylene as above for 7 or 14 d before separating and freezing.

RNA Preparation

Total RNA was prepared from pericarp tissue as previously described (Lashbrook et al., 1994) by powdering frozen tissue in a coffee grinder with a chip of dry ice and then blending with 3 volumes of phenol:chloroform:1 M Tris-HCl (1:1:2, v/v), pH 9.0, using a tissue homogenizer. RNA was precipitated with ethanol, dissolved in 0.1 M Tris-HCl, pH 8.0, containing 0.1% (w/v) SDS, and precipitated in 2 M $LiCl_2$. Contaminating carbohydrates were removed by two precipitations with 33% (v/v) ethanol. RNA was then concentrated by precipitation with ammonium acetate and ethanol and dissolved in diethyl pyrocarbonate-treated water.

Locule and seed samples were freeze-dried before RNA was prepared, added to phenol/chloroform/Tris-HCl as above, and then mixed by vortexing. Seeds were removed by filtration through Miracloth (Calbiochem) and then material blended in a tissue homogenizer. RNA was subsequently prepared as for pericarp, except that only one low-concentration ethanol precipitation of contaminating carbohydrates was performed.

Pericarp RNA from cv Ailsa Craig wild type or *rin* fruit that had been ripened in air or in propylene for 0 to 20 d (Giovannoni et al., 1989) was the kind gift of Dr. Robert L. Fischer (University of California, Berkeley). Fruit were harvested 35 d after anthesis as described above, at which point fruit were at the MG stage, and held in either air or in 500 μL L^{-1} of the ethylene analog propylene for 3, 7, 11, or 20 d. RNA was prepared from pericarp tissue as described by Giovannoni et al. (1989).

RNA was quantified spectrophotometrically by measuring A_{260}. Ratios of A_{260} to A_{280} were generally in excess of 1.7, and ratios of A_{260} to A_{230} typically exceeded 2.0.

Determination of *Cel1* and *Cel2* mRNA Abundance

Radiolabeled RNA probes were transcribed in the antisense orientation from cDNA clones of *Cel1* (truncated with *Hin*cII, re-ligated, and linearized with *Dra*I) and *Cel2* (truncated with *Cla*I, re-ligated, and linearized with *Ssp*I) in pBluescript (Stratagene), using [α-^{32}P]UTP (3000 Ci $mmol^{-1}$, NEN) and T3 or T7 RNA polymerase (Epicentre, Madison, WI). Tritiated full-length transcripts were synthesized in the sense orientation from linearized *Cel1* and *Cel2* subclones in pBluescript using [5,6-^{3}H]UTP (13 Ci $mmol^{-1}$, NEN) and T3 or T7 RNA polymerase and used in the construction of standard curves. *Cel1* or *Cel2* mRNA abundance in 5- or 10-μg aliquots of total RNA was determined using RNase protection assays (RPA II kit; Ambion, Austin, TX). RNase digestion was performed for 30 min at 30°C for *Cel1* and at 37°C for *Cel2* as previously described (Lashbrook et al., 1994). Gels of protected fragments were fixed in 10% acetic acid and 15% ethanol for 1 h and then dried on paper using a gel dryer. Dried gels were exposed to preflashed film (X-Omat AR, Kodak) at −80°C for appropriate lengths of time. mRNA abundance was quantified against the standard curves by exposing dried gels containing digested samples to phosphorimager plates, which were then scanned with a phosphorimager (Fujix BAS 1000; Fuji Medical Systems, Stamford, CT). Analysis of the resulting scans was performed (MacBAS version 1.0 software, Fuji). Relative radioactivities were converted to percentages of mRNA abundance using the standard curve and assuming that mRNA was 3% of total RNA. All assays of *Cel1* or *Cel2* mRNA abundance were performed twice, with very similar results, and are expressed as percentages of mRNA.

RESULTS

Accumulation of *Cel1* and *Cel2* mRNA in Pericarp and Locules

The accumulation of *Cel1* and *Cel2* mRNAs was assayed throughout fruit development using RNase protection assays (Figs. 1 and 2). During the early stages of development (5, 10, and 20 mm) the whole fruit were analyzed, because

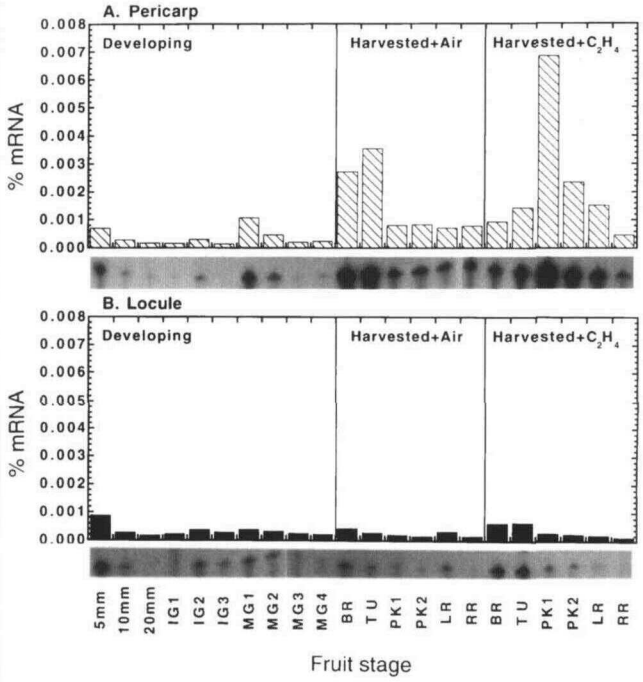

Figure 1. Abundance of *Cel1* mRNA in total RNA derived from pericarp (A) or locule (B) of ripening tomato fruit, *L. esculentum* cv Castlemart. Fruit were collected from the field at defined developmental stages up to MG or ripened from MG off the vine in air or in 10 μL L^{-1} ethylene to the indicated ripening stage. Pericarp and locule material were frozen separately for subsequent preparation of total RNA. *Cel1* mRNA abundance was determined against a standard curve using RNase protection assays and is expressed as a percentage of mRNA present.

they were too small to be separated into pericarp and locule. In subsequent ripening stages (IG–RR), pericarp and locule tissues were analyzed separately.

Cel1 mRNA accumulated transiently in the earliest developmental stage (5-mm-diameter fruit) and reappeared transiently in pericarp at the earliest MG stages (Fig. 1A). The decline in *Cel1* mRNA in later MG stages (MG2–MG4) was consistently observed before mRNA accumulated again at the BR stage, reaching its maximum level at the TU stage in air-treated fruit and then declining. Ethylene treatment slightly stimulated the accumulation of *Cel1* mRNA, with the highest levels of mRNA detected at the PK stage of ripening. In locular tissue, *Cel1* mRNA abundance was low but detectable at all stages of fruit development (Fig. 1B). *Cel1* mRNA in locules did not increase during fruit ripening and its accumulation was not significantly affected by ethylene treatment.

Cel2 mRNA exhibited a pattern of accumulation that was distinct from that of *Cel1* (Fig. 2). *Cel2* mRNA also accumulated transiently at the early stages of fruit development (Fig. 2A), increasing from the 5-mm stage to the 10-mm stage and then declining at the 20-mm stage, but was then completely absent in pericarp and locule tissues until the onset of ripening. *Cel2* mRNA abundance increased greatly at the BR stage and accumulated progressively in the pericarp throughout ripening, achieving at least 2-fold higher levels than *Cel1*, as previously described (Lashbrook et al., 1994). Ethylene treatment accelerated the accumulation of *Cel2* mRNA but only slightly increased the maximum abundance. Unlike *Cel1*, *Cel2* mRNA also accumulated in locules during ripening (Fig. 2B) at levels similar to that observed in pericarp but with a slightly different developmental pattern. *Cel2* mRNA in locules reached its maximum level in BR fruit and then declined progressively throughout ripening. Ethylene treatment had little effect on the maximal accumulation of *Cel2* mRNA in locular tissue.

Cel1 and *Cel2* mRNA Levels in Normal and Ripening-Impaired Mutant Fruit

The data presented above indicate that both *Cel1* and *Cel2* mRNA levels are ripening induced in pericarp tissue and that *Cel2* mRNA also accumulates in the locule. This suggests that these two genes may play a role in the ripening process. To further assess the potential role of each EGase in fruit ripening, the accumulation of *Cel1* and *Cel2* mRNA was assayed throughout fruit ripening in the pericarp of wild-type tomato (cv Ailsa Craig) and compared with mutant *rin* cv Ailsa Craig (Fig. 3). The times indicated in Figure 3 corresponded in wild-type fruit to MG (T0), BR (3 d), PK (7 d), RR (11 d), and overripe (20 d) fruit. Since *rin* fruit ripen extremely slowly, comparisons to wild type were made at the same chronological age rather than at similar ripening stages. Both *Cel1* and *Cel2* mRNA accumulated in air-ripened wild-type cv Ailsa Craig fruit (Fig. 3) at levels similar to those observed in cv Castlemart (Figs. 1 and 2), with *Cel2* being more abundant and showing a maximal accumulation after 11 d. Propylene (used here as an ethylene analog) treatment accelerated the timing of

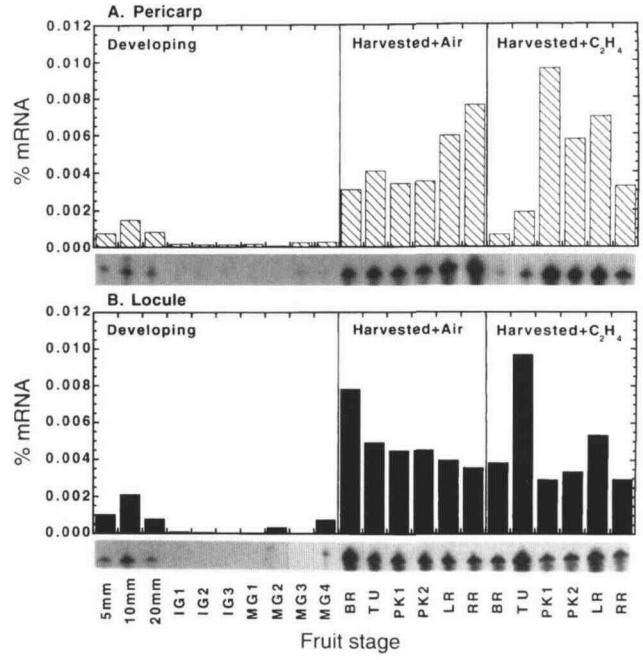

Figure 2. Abundance of *Cel2* mRNA in total RNA derived from pericarp (A) or locule (B) of ripening tomato fruit, *L. esculentum* cv Castlemart. Stages and experimentaal conditions are used in Figure 1.

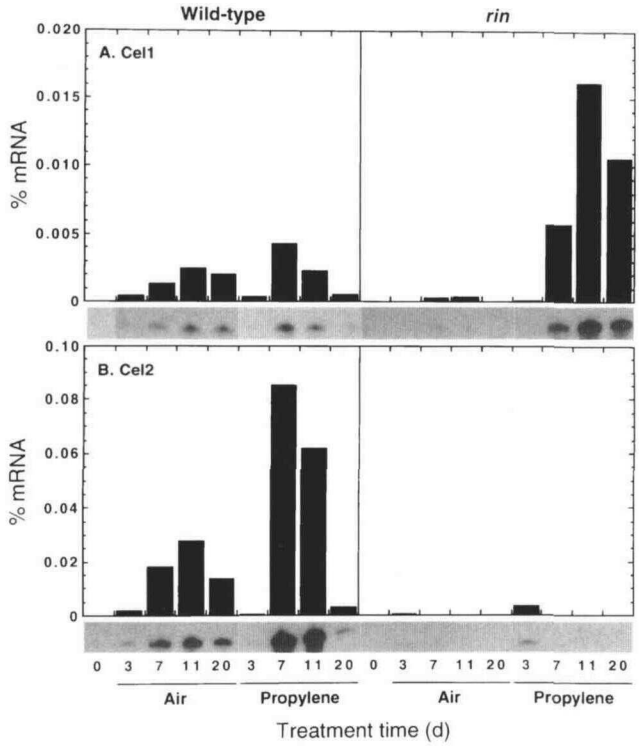

Figure 3. Abundance of *Cel1* (A) and *Cel2* (B) mRNA in total RNA derived from pericarp of ripening fruit of tomato, *L. esculentum* cv Ailsa Craig, either wild type or nearly isogenic for the *rin* ripening mutation. Fruit were harvested 35 d after anthesis (T0) and held in air or in 500 μL L^{-1} propylene for the indicated time. *Cel1* or *Cel2* mRNA abundance in total RNA was determined using RNase protection assays and is expressed as a percentage of mRNA present.

accumulation of both mRNAs, but the maximal level was significantly increased only for *Cel2* mRNA. In *rin* fruit assayed at the same chronological age as for wild-type fruit, *Cel1* mRNA was detectable at very low levels (Fig. 3A) and *Cel2* mRNA was almost completely absent (Fig. 3B) in fruit held in air. However, propylene treatment strongly stimulated the accumulation of *Cel1* mRNA, which reached considerably higher levels than those observed in wild-type fruit. *Cel2* mRNA abundance, however, was only slightly promoted by propylene and remained undetectable except for a very low accumulation after 3 d of propylene treatment. Thus, propylene induction of *Cel1* and *Cel2* expression is differentially blocked by the *rin* mutation, with only *Cel2* expression being inhibited.

The accumulation of *Cel1* and *Cel2* mRNA was also examined in fruit held in the presence or absence of 10 μL L^{-1} ethylene in *rin* and in another ripening mutant, *Nr*. In each case, accumulation of *Cel1* and *Cel2* mRNA was assayed in both pericarp and locule tissue. Relative to wild-type cv Ailsa Craig fruit, the abundance of *Cel1* mRNA was slightly reduced in pericarp tissue of *rin* fruit held in air, and its accumulation was stimulated by ethylene in both pericarp and locule (Fig. 4). In the *Nr* mutant, *Cel1* mRNA was present in pericarp and locule at levels approximately similar to wild-type fruit, although maximal levels were delayed and occurred at 14 rather than at 7 d. In *Nr* pericarp and locule, *Cel1* mRNA levels were not significantly affected by ethylene treatment.

In contrast to *Cel1* mRNA accumulation, which remained ethylene responsive in *rin* fruit, *Cel2* mRNA was completely absent in the pericarp and locule of *rin* mutant fruit held in either air or ethylene (Fig. 5), confirming the results observed using propylene as an ethylene analog (Fig. 3B). *Cel2* mRNA levels were also reduced in both the pericarp and locule of *Nr* mutant fruit and were not significantly affected by ethylene treatment.

DISCUSSION

During early fruit development, *Cel1* and *Cel2* mRNA accumulates transiently to low levels but shows distinct temporal patterns of accumulation (Figs. 1 and 2). *Cel1* is expressed in developing fruit at the youngest stage examined, 5 mm in diameter, which is a period of active cell division (Gillaspy et al., 1993), and then declines. In contrast, *Cel2* mRNA shows greater accumulation at the 10-mm stage, corresponding to a period when cell expansion is just beginning (Gillaspy et al., 1993), but is also present at both the 5- and 20-mm stages. In

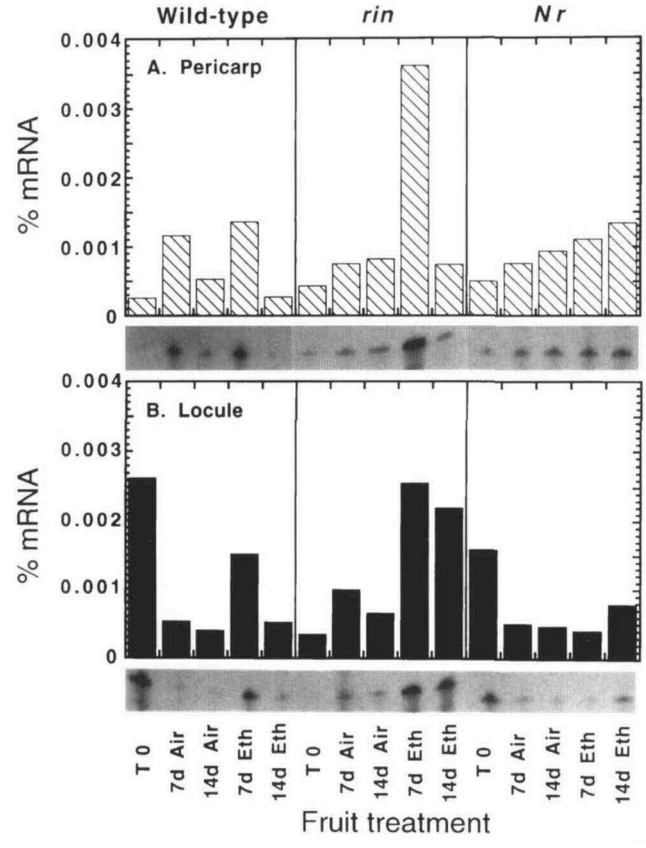

Figure 4. Abundance of *Cel1* mRNA in total RNA derived from pericarp (A) or locule (B) of ripening tomato fruit, *L. esculentum* cv Ailsa Craig, which was either wild type or nearly isogenic for the *rin* or *Nr* ripening mutations. Fruit were harvested 35 d after anthesis (T0) and held in air or in 10 μL L^{-1} ethylene (Eth) for 7 or 14 d. *Cel1* mRNA abundance was determined against a standard curve using RNase protection assays and is expressed as a percentage of mRNA present.

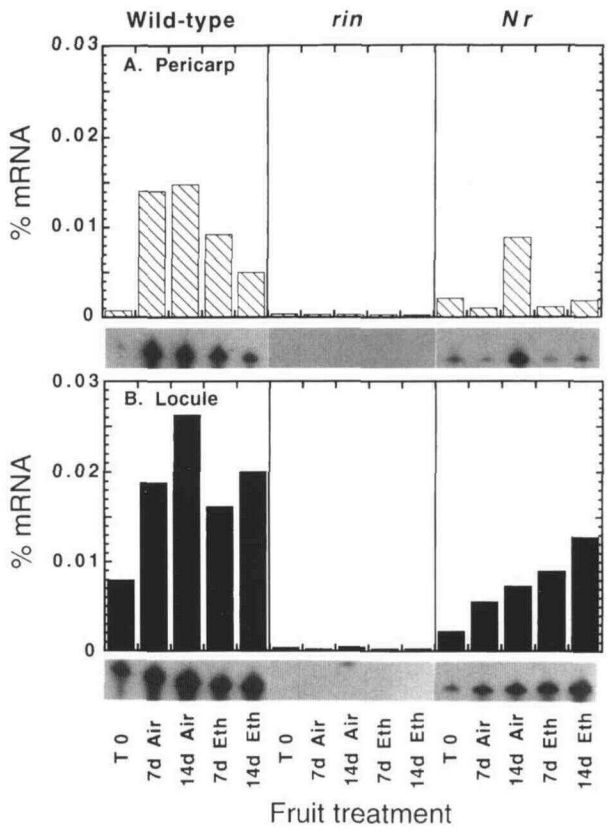

Figure 5. Abundance of *Cel2* mRNA in total RNA derived from pericarp (A) or locule (B) of ripening tomato fruit, *L. esculentum* cv Ailsa Craig, which was either wild type or nearly isogenic for the *rin* or *Nr* ripening mutations. Treatment and experimental conditions are as in Figure 4.

the pericarp, *Cel1* mRNA accumulates transiently in the early stages of MG and then declines, whereas *Cel2* mRNA is present below the level of detection until late MG. This expression in early fruit development suggests that these EGases may participate in processes contributing to changes in cell-wall architecture that accompany tissue growth. The overlapping expression of *Cel1* and *Cel2* mRNA in many developmental contexts may also suggest that the products of these two genes cooperate to bring about changes in cell-wall structure.

Locular gel begins to develop in MG2 and is completely fluid by late MG. Degradation, however, continues, so that by late in ripening locular contents are almost completely liquid (Huber and Lee, 1986). PG activity in locules is low (Huber and Lee, 1986), but considerable EGase activity is present and increases with ripening until the PK or red stages (Huber, 1985; Maclachlan and Brady, 1992). Locular pectins (Huber and Lee, 1986) and cellulose (Maclachlan and Brady, 1994) exhibit only modest depolymerization during ripening, but locular xyloglucan undergoes substantial depolymerization that occurs mainly in red fruit after initial locular gel formation is complete (Huber and Lee, 1986; Maclachlan and Brady, 1994). In locules, neither *Cel1* nor *Cel2* mRNA is detected in significant amounts before MG4, indicating that these gene products do not play a role in initial locule liquefaction, which is essentially complete by MG3. Furthermore, *rin* fruit possess normal, liquefied locules, although *Cel2* fails to be expressed in this tissue (Fig. 5). *Cel2* mRNA levels in locules increase strikingly at the BR stage (Fig. 2B), suggesting that the *Cel2* gene product may contribute to xyloglucan depolymerization and the terminal stages of locule liquefaction occurring after this time.

At approximately the BR stage numerous ripening-related changes are beginning to occur in the fruit, including increased ethylene evolution, climacteric respiration, degradation of chlorophyll, synthesis of lycopene and aromatic compounds, and a disassembly of cell-wall components that eventually leads to a softening of the pericarp tissue (Brady, 1987; Fischer and Bennett, 1991). After the BR stage, a ripening-related increase in cell-wall hydrolases has been described (Fischer and Bennett, 1991), including increases in mRNA abundance and activity of PG (Brady et al., 1982; DellaPenna et al., 1986) and EGases (Hall, 1964; Hobson, 1968; Lashbrook et al., 1994). In pericarp the mRNA abundance of both *Cel1* and *Cel2* increases markedly at the BR stage (Figs. 1 and 2). However, *Cel2* achieves considerably higher mRNA levels than those of *Cel1* and continues to accumulate with ripening, whereas the abundance of *Cel1* mRNA declines after the TU stage. Total extractable EGase activity has been reported to increase during ripening up to the RR stage (approximately 4 d after BR) before declining (Hall, 1964; Sobotka and Watada, 1971). These observations suggest that the *Cel2* gene product contributes more to total EGase activity during ripening than does that of *Cel1*. In fruit ripened in exogenous ethylene, mRNA accumulation in pericarp is only slightly promoted for both *Cel1* and *Cel2*. However, ripening tomato fruit produce considerable endogenous ethylene (Lashbrook et al., 1994), which may be sufficient to virtually saturate ripening responses dependent on ethylene, including induction of *Cel1* and *Cel2* mRNA. In experiments in which the ethylene action antagonist 2,5-norbornadiene was used, it was shown that accumulation of both *Cel1* and *Cel2* mRNAs was ethylene dependent (Lashbrook et al., 1994). These observations suggest that mRNA accumulation of both of these genes is enhanced by ethylene, either directly or indirectly.

The *rin* and *Nr* ripening-impaired mutants are caused by separate single-gene mutations and each causes multiple effects on fruit ripening (Tigchelaar et al., 1978). Whereas the *Nr* gene merely retards the onset of ripening and reduces the magnitude of ripening-related changes, fruit of the *rin* mutant are nonclimacteric, ethylene production is very low, and most ripening-related changes are almost completely inhibited (Tigchelaar et al., 1978). Treatment of fruit with exogenous ethylene does not reverse the impaired ripening phenotypes but does cause a reduced or delayed induction of some ripening-related genes in both *rin* (Lincoln and Fischer, 1988; DellaPenna et al., 1989; Knapp et al., 1989; Picton et al., 1993) and *Nr* (Yen et al., 1995). The *Nr* gene causes ethylene insensitivity in fruit and many other tissues of tomato plants (Lanahan et al., 1994) and is closely linked to a homolog of the Arabidopsis *ETR1* gene (Yen et al., 1995), the product of which may be in-

volved in ethylene perception and signaling (Chang et al., 1993). The study of ripening mutants such as *rin* and *Nr* has provided important insights into the natural regulation of ripening-associated genes. The expression of *E4* and *E8*, two tomato ripening-related genes of unknown function, is significantly promoted by ethylene in wild-type fruit (Lincoln and Fischer, 1988). In *rin* fruit, mRNA of the *E4* gene is present at barely detectable levels and *E8* is much reduced (Lincoln and Fischer, 1988; DellaPenna et al., 1989), but ethylene treatment promotes mRNA accumulation of both genes (Yen et al., 1995). Tomato *Cel1* mRNA shows an expression pattern similar to that of *E4* and *E8* in that it is very low in *rin*, but ethylene strongly increases its abundance (Figs. 3A and 4). In contrast, *Cel2* mRNA accumulation shows many characteristics similar to that of the *PG* gene. Accumulation of *PG* mRNA increases progressively during ripening (DellaPenna et al., 1986) but is virtually absent in *rin* (DellaPenna et al., 1989) even after ethylene treatment (Yen et al., 1995). *Cel2* mRNA accumulation also increases progressively during ripening (Fig. 2) but is absent in *rin*, and its expression in both pericarp and locules is insensitive to propylene or ethylene in this genetic background (Figs. 3B and 5). In *Nr*, mRNA of *E4* is almost absent, whereas *E8* and *PG* show a reduced and delayed accumulation (DellaPenna et al., 1989), and ethylene treatment does not promote mRNA accumulation of any of the three genes (Yen et al., 1995). Similarly, *Cel1* and *Cel2* show a delayed accumulation of mRNA relative to wild type, and the promotions seen in ethylene-treated *Nr* are not significant (Figs. 4 and 5). Thus, the accumulations of mRNA of *Cel1* and *Cel2* during fruit ripening show several similarities, but their expression is not coordinated and the differential effects of the *rin* mutation indicate that they are subject to distinct regulatory control.

Fruit of *rin* as well as transgenic *rin* fruit expressing a *PG* gene fail to soften at wild-type rates even when exposed to the ethylene analog propylene (Giovannoni et al., 1989). The observation that the *Cel1* gene is expressed in propylene- or ethylene-treated *rin* fruit suggests that the product of this gene is also not sufficient to promote ripening-associated cell-wall disassembly or fruit softening. However, the inhibition of expression of the *Cel2* gene by the *rin* mutation, even in the presence of ethylene, suggests that the product of the *Cel2* gene contributes to ripening-associated cell-wall changes.

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